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# Neuronal differentiation of adipose-derived stem cells and their transplantation for cerebral ischemia\*

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## Abstract

**OBJECTIVE:** To review published data on the biological characteristics, differentiation and applications of adipose-derived stem cells in ischemic diseases.

**DATA RETRIEVAL:** A computer-based online search of reports published from January 2005 to June 2012 related to the development of adipose-derived stem cells and their transplantation for treatment of cerebral ischemia was performed in Web of Science using the key words "adipose-derived stem cells", "neural-like cells", "transplantation", "stroke", and "cerebral ischemia".

**SELECTION CRITERIA:** The documents associated with the development of adipose-derived stem cells and their transplantation for treatment of cerebral ischemia were selected, and those published in the last 3–5 years or in authoritative journals were preferred in the same field. Totally 89 articles were obtained in the initial retrieval, of which 53 were chosen based on the inclusion criteria.

**MAIN OUTCOME MEASURES:** Biological characteristics and induced differentiation of adipose-derived stem cells and cell transplantation for disease treatment as well as the underlying mechanism of clinical application.

**RESULTS:** The advantages of adipose-derived stem cells include their ease of procurement, wide availability, rapid expansion, low tumorigenesis, low immunogenicity, and absence of ethical constraints. Preclinical experiments have demonstrated that transplanted adipose-derived stem cells can improve neurological functions, reduce small regions of cerebral infarction, promote angiogenesis, and express neuron-specific markers. The improvement of neurological functions was demonstrated in experiments using different methods and time courses of adipose-derived stem cell transplantation, but the mechanisms remain unclear.

**CONCLUSION:** Further research into the treatment of ischemic disease by adipose-derived stem cell transplantation is needed to determine their mechanism of action.

## Key Words

adipose-derived stem cells; adipose stem cells; differentiation; adipose tissue; neural-like cells; transplantation; stroke; cerebral ischemia; cerebrovascular disease; stem cell therapy

## Research Highlights

(1) Adipose-derived stem cells (ADSCs) are ideal candidates for neural stem cell transplantation.

(2) This study summarizes studies on ADSCs transplantation for cerebral ischemia with respect to the biological characteristics of ADSCs, the expression of surface antigens by ADSCs, the differentiation of ADSCs into nerve cells, stereotaxic heterogenic transplantation of ADSCs, heterogenic intravenous transplantation of ADSCs, and allogeneic intravenous transplantation of ADSCs. The transplantation of ADSC-conditioned medium for the treatment of cerebrovascular disease is also discussed.

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## Abbreviations

ADSCs, adipose-derived stem cells; MSCs, mesenchymal stem cells; EGF, epidermal growth factor; FGF, fibroblast growth factor; MCAO: middle cerebral artery occlusion

## INTRODUCTION

Cerebral ischemia is a common acute cerebrovascular disease. The key to its treatment involves the rescue of dying neurons in the ischemic penumbra and the promotion of recovery from injury. The accepted mechanisms for cerebral ischemia include:  $Ca^{2+}$  overloading, free radical and lipid peroxidation, mitochondrial dysfunction, deregulated expression of nitrogen monoxide, cytokines, immediate early genes, heat shock protein, and cell apoptosis. Cerebral ischemia is associated with high levels of mortality and disability, and current medical therapy for cerebral ischemia patients is limited. Most patients primarily receive supportive treatment<sup>[1]</sup> and only selected patients are subjected to surgical treatment<sup>[2]</sup>. Neural cell replacement therapy is the only effective treatment. Neural cell transplantation can rebuild nerve conduction loops and restore some neurological function, possibly *via* the differentiation of transplanted stem cells into functional glial cells or neurons, which can thus substitute for some of the affected neurons. The transplanted stem cells secrete cytokines to improve the local microenvironment of ischemic necrosis in terms of inflammation, tissue necrosis and glial scarring<sup>[3-4]</sup>. However, cerebral ischemic damage can affect large areas and many kinds of nerve cells. Ischemic lesions may involve a number of sites in the hypothalamus, striatum, hippocampus and cortex, and reconstruction of this complex system presents a challenge for cell transplantation. Mesenchymal stem cells (MSCs) differ from hematopoietic stromal cells in that they are pluripotent precursor cells. Adipose-derived MSCs (ADSCs) are similar to marrow stromal cells, and offer advantages such as strong amplification, convenience and availability, and lack of immune rejection. As such, ADSCs have proven to be a feasible and effective cell source for neural cell replacement therapy in cerebral ischemia<sup>[5-6]</sup>.

## DATA SOURCES AND METHODOLOGY

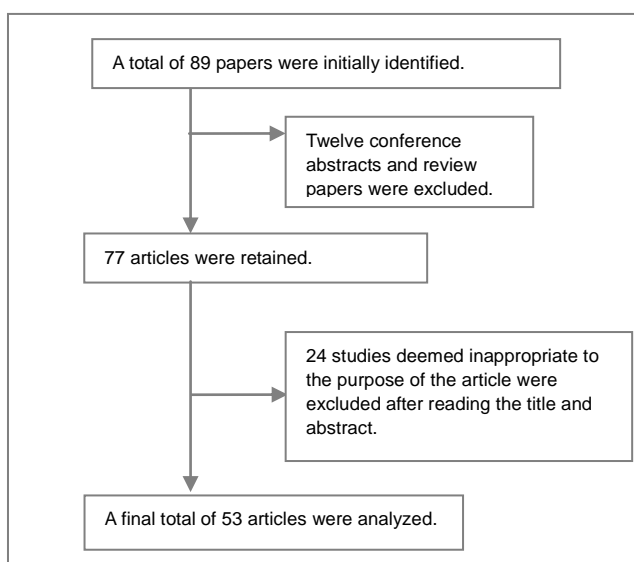
### Data retrieval

A computer-based online search of original reports published from January 2005 to June 2012 related to the development of ADSCs and their transplantation for treatment of cerebral ischemia was performed in Web of Science using the key words “adipose-derived stem cells”, “adipose stem cells”, “differentiation”, “neural-like cells”,

“transplantation”, “brain stroke”, and “cerebral ischemia”.

### Selection criteria

The documents associated with the development of ADSCs and their transplantation for treatment of cerebral ischemia were selected, and those published in the last five years or in authoritative journals were preferred in the same field. Repetitive studies were excluded from this review. 89 articles in English and Chinese were obtained in the initial retrieval, of which 53 were chosen for review based on the inclusion criteria. A flow chart summarizing the literature screening process is shown below:



## RESULTS

### Biological characteristics of ADSCs

In 2001, researchers from the University of California Los Angeles and the University of Pittsburgh first reported that stem cells could be isolated from adipose tissue removed during liposuction<sup>[7]</sup>. Since then, several groups have reported the isolation of stem cells from adipose tissues by similar isolation methods<sup>[8-9]</sup>. Recently, researchers have suggested that ADSCs can be differentiated into new fat tissue, bone, cartilage, nerve, muscle and endothelial cells<sup>[10-11]</sup>. In animal studies, these cells show potential for the treatment of heart attack, stroke, bone injury, and even neurological diseases<sup>[12-13]</sup>. ADSCs have been referred to as preadipocytes, mesenchymal cells, treated liposuction cells, multi-potential adipose-derived stem cells and adipose-derived adult stromal cells, and were named adipose-derived stem cells at the 2<sup>nd</sup> annual meeting of the

International Fat Applied Technology Society in 2004<sup>[14-15]</sup>.

### Expression of surface antigens by ADSCs

ADSCs possess the same characteristics as MSCs<sup>[16-19]</sup>, including (1) adhesion in routine culture conditions; (2) the potential to differentiate into bone, fat and cartilage; (3): a similar morphology to fibroblasts, with adhesive polymeric colony-forming units; (4) positive expression of Stro-1, CD13, CD29, CD44, CD63, CD73, CD90 and CD166. The expression of these markers is low during the early stages of culture and increases significantly with increasing passage number<sup>[20-21]</sup>; (5) negative expression of hemopoietic stem cell markers such as c-kit, CD11b, CD14, CD19, CD34, CD45, CD79a and HLA-DR. In general, the markers for ADSCs are different in each experiment, possibly because of different culture methods, different cell passage numbers, distinct immunohistochemical susceptibility or diverse monoclonal antibodies. Currently, there are no specific surface markers for adipose-derived stem cells. In experiments to characterize ADSCs, 3–5 representative positive or negative markers are selected to identify the ADSCs. Flow cytometry in particular, and also immunohistochemistry, are commonly used to identify ADSCs.

### Induced differentiation of ADSCs into nerve cells

With the maturation of techniques for the *in vitro* differentiation of ADSCs, researchers have investigated whether ADSCs can survive and differentiate *in vivo*. Kopen *et al*<sup>[22]</sup> injected bone marrow stromal stem cells into the lateral cerebral ventricles of mice, and 12 days later the cells had migrated to the olfactory bulb, procerbrum and cerebellum, and some cells had migrated into the corpora striata and stratum moleculare hippocampi where they expressed glial fibrillary acidic protein. Other cells migrated to the reticular formation of the brain stem and expressed NF-70. This migration is similar to the process of postnatal neural development, which indicates that as neural precursor cells, bone marrow stromal stem cells can migrate in the brain following transplantation into the lateral cerebral ventricle and differentiate into glioblasts and neurons. Safford *et al*<sup>[23]</sup> injected induced ADSCs into the hippocampal CA1 region and found that the transplanted ADSCs migrated along the corpus callosum and corpus striatum from the mouth and tail shaft for up to 2 mm, and survived for 12 weeks. The induced ADSCs resembled nerve cells due to the expression of neurone specific enolase and NeuN. However, ADSCs that were not induced *in vitro* did not exhibit these behaviors, indicating that *in vitro* induction of ADSCs is essential for their survival in the microenvironment after transplantation.

Many factors influence the neural differentiation and migration of ADSCs, such as the chemokines epidermal growth factor (EGF), fibroblast growth factor 2 (FGF2), insulin-like growth factor, platelet-derived growth factor, brain-derived neurotrophic factor and nerve growth factor, which have been demonstrated to promote proliferation and neurogenesis in stem cells in the mammalian brain. EGF is the most potent stimulator of neurogenesis in neural stem cells in the lateral cerebral ventricle. When EGF and fibroblast growth factor 2 were injected into the lateral cerebral ventricles of rats, excitatory nerve stem cell hyperplasia increased 5–7-fold, accompanied by prolonged life spans. EGF-dependent lateral ventricular neurogenesis and hyperplasia are mediated by the EGF/EGFR signaling pathway<sup>[24]</sup>. EGF can induce proliferating neural precursor cells in the lateral cerebral ventricle to migrate to the surrounding brain tissue (including corpus striatum), but only a few cells differentiate into neuron-like cells<sup>[25]</sup>. Other reports of neurogenic differentiation also demonstrated that ADSCs exhibit a neuron-like morphology and express several proteins and genes consistent with the neuronal phenotype<sup>[26-30]</sup>.

### ADSCs for the treatment of ischemic cerebrovascular disease

Cerebrovascular disease is a common ailment that is responsible for a very high number of deaths. Cerebrovascular disease can be divided into ischemic stroke and hemorrhagic stroke according to the pathological changes, and the former occurs at a high frequency. At present, ADSCs have been applied in orthopedics. Clinical research has confirmed that ADSCs possess many advantages, including their ease of procurement, wide availability, rapid expansion, low tumorigenesis, low immunogenicity, and absence of ethical constraints. Thus, ADSCs provide a new approach for the treatment of cerebrovascular disease.

### Stereotaxic heterogenic transplantation of ADSCs for the treatment of cerebrovascular disease

Studies have demonstrated that transplantation of ADSCs can induce endogenous neural stem cells to proliferate, differentiate and migrate to improve the recovery of neural functions in rats with cerebrovascular disease (Table 1).

### Heterogenic intravenous transplantation of ADSCs for the treatment of cerebrovascular disease (Table 2)

### Allogeneic intravenous transplantation of ADSCs for the treatment of cerebrovascular disease (Table 3)

Table 1 Studies on stereotaxic heterogenic transplantation of adipose-derived stem cells (ADSCs) for the treatment of ischemic cerebrovascular disease

Author	Method	Result	Conclusion
Chen <i>et al</i> <sup>[31]</sup>	ADSCs were labeled <i>in vitro</i> with superparamagnetic iron oxide and poly-L-Lysine and transplanted stereotactically into the contralateral cerebral hemisphere to investigate the distribution and migration of ADSCs in a rat model of ischemia brain injury. Ligation of the middle cerebral artery under a microscope was used to cause focal brain infarction in rats.	A significant improvement in neurological severity score was observed in rats receiving transplanted ADSCs compared with the control group after 3 weeks ( $P < 0.05$ ). The transplanted ADSCs preferentially migrated toward the injured brain through the callosum. Neurological and functional improvement was observed in rats that received transplanted ADSCs following ischemic brain injury.	ADSCs can be directly induced to differentiate into neural-like cells.
Kang <i>et al</i> <sup>[32]</sup>	Human adipose tissue stromal cells (hATSCs) labeled with LacZ adenovirus were injected into the lateral ventricle of the rat brain to treat middle cerebral artery occlusion (MCAO).	Transplanted cells migrated to various parts of the brain and differentiated into neuron-like cells that expressed MAP2 and GFAP. Ischemic brain injury by MCAO increased their migration to the injured cortex. Transplantation of BDNF-transduced hATSCs significantly improved motor recovery of functional deficits in MCAO rats.	hATSCs showed good histocompatibility with rat brain tissues, and no local neoplastic hyperplasia could be found. The transplanted hATSCs survived, migrated, and improved functional recovery after stroke, and genetically engineered hATSCs expressed biologically active gene products.

Table 2 Studies on heterogenic intravenous transplantation of human adipose-derived stem cells (hADSCs) for the treatment of cerebrovascular disease

Author	Method	Result	Conclusion
Chu <i>et al</i> <sup>[33]</sup>	Chronic cerebral hypoperfusion was induced using the two-vessel occlusion model by permanent ligation of the bilateral carotid arteries in rats. hADSCs ( $2 \times 10^6$ /mL) were intravenously injected into rats at 1 day, 1 week and 3 weeks respectively. A control group and a sham-surgery group were also included. Behavioral studies were performed 5 weeks after BCAL to observe the effects of hADSCs at different time points on cognitive impairment induced by chronic cerebral hypoperfusion in rats.	The water maze test showed that the latent period was not statistically significant between the hADSCs group and the control group at 1 day and 1 week ( $P > 0.05$ ), but a significant difference was found between the two groups at 3 weeks. In addition, the effects of hADSCs on chronic cerebral hypoperfusion in rats were time dependent, and the cognitive impairment of chronic cerebral hypoperfusion in acute phase rats (1 day and 1 week groups) was obviously improved compared with the chronic phase (3 week group).	Intravenous infusion of hADSCs can improve the cognitive impairment of chronic cerebral hypoperfusion in rats, and the effect of the chronic phase is better than the acute phase.
Yang <i>et al</i> <sup>[34]</sup>	hADSC were injected <i>via</i> a right femoral vein to treat rats inflicted with experimental intracerebral hemorrhage.	Behavioral analysis of the experimental animals seven days after the hADSC therapy showed a significant functional improvement in the rats with stem cell therapy compared with the rats in the control group without stem cell therapy. The injected hADSCs were detectable in the brains of the hADSC treated rats, as determined by histochemical analysis.	The infused hADSCs facilitated functional recovery in the experimental animals with intracerebral hemorrhage-induced stroke.
Kim <i>et al</i> <sup>[35]</sup>	Intracerebral hemorrhage was induced <i>via</i> the stereotaxic infusion of collagenase, and hADSCs (three million cells per animal) were intravenously administered at 24 hours post-intracerebral hemorrhage induction.	The results showed that brain water content, TUNEL <sup>+</sup> , and MPO <sup>+</sup> cell numbers were significantly reduced in the hADSC-transplanted rats. hADSC transplantation attenuated neurological deficits from 4 to 5 weeks post- intracerebral hemorrhage, and reduced both the brain atrophy and the glial proliferation at 6 weeks. Transplanted hADSC were found to densely populate the perihematomal areas at 6 weeks, and to express endothelial markers, but not neuronal or glial markers.	hADSC transplantation in the intracerebral hemorrhage model reduced both acute cerebral inflammation and chronic brain degeneration, and promoted long-term functional recovery.

Table 3 Studies on allogeneic intravenous transplantation of adipose-derived mesenchymal stem cells (ADSCs) for the treatment of cerebrovascular disease

Author	Method	Result	Conclusion
Leu <i>et al</i> <sup>[36]</sup>	Thirty rats underwent homolateral middle cerebral artery occlusion and were divided into a control group (at 0, 12 and 24 hours after model induction) and a treatment group ( $2.0 \times 10^6$ intra-venous ADMSCs) after occlusion of the distal left internal carotid artery. ADMSCs were labeled with CM-Dil <i>in vitro</i> . The rats were sacrificed and brain tissues were harvested on day 21 after the procedure.	The results showed that the brain infarction area was smaller in the treatment group than in the control group ( $P < 0.05$ ). The corner test identified a higher frequency of turning movement to the left in the control group than in the treatment group. Western blotting showed higher expression of CXCR4 and stromal-cell derived factor-1 in the treatment group ( $P < 0.01$ ). Western blotting demonstrated lower CXCR4 and stromal-cell derived factor-1 (SDF-1) expression in the control group than in the treatment group ( $P < 0.01$ ). Immunofluorescence staining showed lower expression of CXCR4, SDF-1, von Willebrand factor and doublecortin, whereas the number of apoptotic nuclei on TUNEL assay was higher in the control group than in the treatment group ( $P < 0.01$ ). Immunohistochemical staining showed that cell proliferation and the number of small vessels was lower but glial fibrillary acid protein was higher in the control group than in the treatment group ( $P < 0.01$ ).	ADMSCs migrated and improved sensorimotor dysfunction and promoted neogenesis and blood supply in the focal zone. Additionally, ADMSCs exhibited anti-inflammatory and anti-apoptotic effects, and expressed neuron-specific markers.
Yang <i>et al</i> <sup>[37]</sup>	ADSCs were isolated from rat adipose tissue and then induced to initiate neural differentiation. Subsequently, rat ADSCs were transplanted into rats for the treatment of cerebral ischemia.	Following neural induction, ADSCs developed a neural morphology and expressed Nestin, MAP2 and GFAP. The neurobehavioral function, infarct volume and cell properties such as apoptosis, survival, migration, proliferation, differentiation and immunogenicity were analyzed. Treatment with ADSCs results in better functional recovery and greater reduction in hemispheric atrophy compared with controls.	ADSCs therapy is promising in stroke treatment and provides efficacious therapeutic modalities with much better outcome in clinical patients.
Ikegame <i>et al</i> <sup>[38]</sup>	ADSCs or BMSCs were administrated intravenously into recipient mice ( $1 \times 10^5$ cells/mouse) immediately after reperfusion following a 90-min middle cerebral artery occlusion.	<i>In vitro</i> conditions allowed ADSCs to differentiate into neural, glial and vascular endothelial cells. ADSC administration showed remarkable attenuation of ischemic damage, although the ADSCs were not fully incorporated into the infarct area. Nonetheless, the expression of HGF and angiotensin-1 in ischemic brain tissue was significantly increased in ADSC-treated mice compared with the BMSC group.	Compared with BMSCs, ADSCs have great advantages for cell preparation because of easier and safer access to adipose tissue, suggesting that ADSCs would be a more preferable source for cell therapy for brain ischemia than BMSCs.

### Transplantation of ADSCs-conditioned medium (CM) for the treatment of cerebrovascular disease

The use of stem cell CM instead of direct implantation of stem cells is a feasible approach to overcoming the limitations of current cell-based therapy. Many cytokines and growth factors such as granulocyte-macrophage colony-stimulating factor, vascular EGF, hepatocyte growth factor, basic FGF (bFGF), transforming growth factor and insulin-like growth factor-1 have been identified in the CM of various stem cells<sup>[39-46]</sup>, which may be responsible for the paracrine protective effects of stem cells against various cytotoxic insults. Previous studies have reported the use of stem cell CM for experimental regenerative therapies. For example, CM obtained from amniotic fluid-derived MSCs and ADSCs significantly enhanced wound healing<sup>[47]</sup>. Endothelial progenitor cell CM induced neovascularization in a rat hindlimb

ischemia model<sup>[48]</sup>. Cho *et al*<sup>[49]</sup> reported that continuous infusion of CM or aMEM medium (0.5 L/h) into the lateral ventricle that was initiated 8 days after surgery and maintained for 7 days induced significant functional and structural recovery after stroke as a consequence of enhanced neovascularization, reduced neural cell apoptosis, and milder astrogliosis. In Egashira's study<sup>[50]</sup>, intracerebroventricular administration of 30- and 100-fold concentrated murine ASC-CM 1 hour prior to middle cerebral artery occlusion (MCAO) resulted in a dose-dependent reduction in the infarct volume and the brain swelling. The administration of murine ASC-CM immediately after MCAO was also effective, but administration 2 hours after MCAO was not. Pretreatment with 100-fold concentrated murine ASC-CM at 10% of the total culture volume significantly reduced glutamate-induced excitotoxicity in SH-SY5Y cells. A similar reduction in the MCAO-induced infarction volume

was seen following administration of 100-fold concentrated human ASC-CM or murine ASC-CM. These results demonstrated that ASC-CM appears to promote recovery after experimental ischemic stroke in both *in vivo* and *in vitro* models. These findings suggest the feasibility of ASC-CM administration as a therapy for acute stage stroke.

### Mechanisms of action of transplanted ADSCs in the treatment of cerebrovascular disease

A deeper understanding of the molecular mechanisms underlying the differentiation of ADSCs would facilitate the application of ADSC transplantation in the treatment of cerebrovascular disease. miRNAs can regulate gene expression by inhibiting mRNA translation or promoting mRNA degradation. Previous studies have shown that miRNAs are highly correlated with stem cell self-renewal and differentiation, and play important roles in regulating stem cell activity<sup>[51-52]</sup>. miRNA-125 has been described as a key regulatory molecule of the bioactivities of neural stem cells<sup>[53]</sup>, and several miRNAs have been shown to maintain the stem cell phenotype and induce directional differentiation<sup>[54]</sup>. For example, miR-138 expression is downregulated during differentiation of ADSCs into adipocytes, and inhibition of EP300-interacting inhibitor of differentiation 1 can suppress this differentiation<sup>[55]</sup>. Furthermore, high miR-184 expression can promote the proliferation of neural stem cells and inhibit their differentiation<sup>[56]</sup>.

The following four routes may describe the underlying mechanisms of action of ADSCs in the treatment of cerebrovascular disease. (1) ADSCs substitute for absent neural cells via differentiation into neurons and glial cells; (2) ADSCs promote neovascularization and improve focal blood supply by secreting various vascular growth factors and differentiating into vascular endothelial cells; (3) ADSCs facilitate functional recovery by secreting neurotrophic factors; and (4) ADSCs promote self-repair by stimulating endogenous neural stem cells to differentiate and mature.

### CONCLUSION

ADSCs have many potential advantages for clinical applications. However, there are still many problems to be solved to enable the clinical application of ADSCs. Various factors affect the differentiation of ADSCs into nerve cells and the mechanisms are not fully understood. Additionally, it is still not clear whether the induced neuron-like cells or glial-like cells exhibit nerve cell

functions. Further nerve electrophysiology and neural biochemical experiments are needed to confirm this. At present, the biological activities of ADSCs are well-characterized *in vitro*, but their regulation by various factors in body fluids *in vivo* is unclear. Future research should focus on: (1) *in vivo* experiments, changes in gene expression that induce differentiation, and ensuring the stability of recombinant genes in expanded cell populations; (2) avoiding immunological rejection in clinical applications and immune disorders after transplantation *in vivo*; (3) excluding infectivity and the potential for carcinogenesis. It is currently unknown whether stem cell transplantation can improve function in stroke patients by replacing the damaged cells and reconstructing the neural circuits. Therefore, the key issues to be solved regarding stem cell transplantation for the treatment of stroke are how to improve the differentiation of transplanted stem cells into specific neurons and glial cells, and their integration into host brain structure and function.

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**Author contributions:** Guoping Tian integrated data, conceived and designed this study. Jin Zhou wrote the draft of the manuscript and contributed to the evaluation of the study. Jing'e Wang, Bing Xu, Li Li, Feng Zhu, Jian Han and Jianping Li retrieved the references, extracted the data, and provided technical support. Siyang Zhang analyzed data. Guoping Tian revised the manuscript and was responsible for funding.

**Conflicts of interest:** None declared.

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